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Sustainable organic substrate production using millicompost in combination with different plant residues for the cultivation of *Passiflora edulis* seedlings

Luiz Fernando de Sousa Antunes [a,*], André Felipe de Sousa Vaz [a], Luiz Aurélio Peres Martelleto [a], Marco Antonio de Almeida Leal [b], Renata dos Santos Alves [a], Talita dos Santos Ferreira [a], Norma Gouvêa Rumjanek [b], Maria Elizabeth Fernandes Correia [b], Raul Castro Carriello Rosa [b], José Guilherme Marinho Guerra [b]

[a] *Universidade Federal Rural do Rio de Janeiro, Seropédica, Rodovia BR 465, Km 07., Rio de Janeiro, Zip Code 23897-000, Brazil*
[b] *Embrapa Agrobiologia, Rodovia BR 465, km 7, 23.897-000, Seropédica, Rio de Janeiro, Zip Code 23891-000, Brazil*

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A B S T R A C T

The use of sustainable organic substrates of recognized quality is essential in the production of seedlings of fruit species. In this context, the present study aimed to formulate different sustainable organic substrates based on millicompost combined with different proportions of locally available organic waste, such as coconut fiber (*Cocos nucifera*) powder, gliricidia (*Gliricidia sepium)* and elephant grass shavings (*Pennisetum purpureum)* for the production of *Passiflora edulis* seedlings. The organic substrates used were as follows: S1- millicompost; S2- commercial substrate and substrates formulated based on millicompost: S3%–25% millicompost + 25% gliricidia + 25% elephant grass + 25% coconut fiber; S4%–50% millicompost+ 50% elephant grass; (S5) 50% millicompost+ 50% gliricidia; (S6) 50% millicompost+ 50% coconut fiber. At 56 days of sowing, the following morphological parameters were evaluated: shoot dry mass (SDM); root dry mass (RDM); plant height (PH); number of leaves (NL); stalk diameter (SD); root volume (RV); leaf area in cm^2 (LA); seedling vigor (SV) and clod stability (CS). The nutrient contents (N, P, K, Ca and Mg) accumulated in the shoot dry mass of the seedlings were determined after this period. The organic substrates that provided seedlings with significantly higher parameters ($P < 0.05$) of SDM, RDM, PH, NL, SD, RV, LA, SV and CS were as follows: S1> S6> S5. The other substrates (S2, S3 and S4) presented seedlings with morphological parameters similar to each other. In addition, there was a greater accumulation of P, K Ca and Mg ($P < 0.05$) in the shoot dry mass of the seedlings developed on the substrates S1> S6> S5. The substrates S6 and S5 represent two new options of sustainable substrates, being effective in the production of yellow passion fruit seedlings with excellent vegetative growth rates.

* Correspondence to: Universidade Federal Rural do Rio de Janeiro, Seropédica, Rodovia BR 465, Km 07., Rio de Janeiro, Zip Code 23890-000, Brazil.
E-mail addresses: fernando.ufrrj.agro@gmail.com (L.F.d.S. Antunes), afsagro@hotmail.com (A.F.d.S. Vaz), luizmarte@hotmail.com (L.A.P. Martelleto), marco.leal@embrapa.br (M.A.d.A. Leal), renata_9rj@hotmail.com (R.d.S. Alves), talirafalau@gmail.com (T.d.S. Ferreira), norma.rumjanek@embrapa.br (N.G. Rumjanek), elizabeth.correia@embrapa.br (M.E.F. Correia), raul.rosa@embrapa.br (R.C.C. Rosa).

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1. Introduction

Brazil stands out in fruit production with commercial production of numerous fruit trees, including yellow passion fruit (*Passiflora edulis* Sims f. *flavicarpa* Deg.). Among the most important, the *Passiflora* genus gathers approximately 400 species, which 120, hitherto cataloged, are native to Brazil. In addition, Brazil is a world leader in the production and consumption of passion fruit, favored by climate and water conditions that contribute to the development of this culture (Borges et al., 2019).

According to the Agricultural Census from 2018, 602,651 tons of passion fruit were harvested in Brazil in 42,731 hectares, thus estimating an average yield per hectare of 14.10 tons. The value of production reached 1,014,599 million reais, being Northeast region the most expressive with 58.37%, followed by the Southeast (18.10%), South (11%), North (8.55%) and Center regions-West (3.96%) (IBGE, 2018). To obtain a uniform plant stand, it is essential to use good quality seedlings, because seedlings with inferior quality compromise the final plant performance, leading to damage in production and delaying the production cycle (Costa et al., 2018). Considering that the seedlings production has two essential criteria: the cost of acquisition and the availability of material for the substrates production (Meneghelli et al., 2017). The substrate quality has great importance and must have adequate physical, physical–chemical, chemical, and biological properties to provide the necessary nutrients for the full development of the plant (Kato et al., 2018; Antunes et al., 2019).

World population is unlikely to stop growing this century. In this point of view, the planet's intensive farming practices are applied to produce more food. This also promotes the rapid increase in the volume and diversity of residues from agricultural biomass, representing, in a global scale, 140 billion tons each year (Singhania et al., 2017). Therefore, promoting the use of agricultural residues available on farms as substrates for the seedlings production represents an economically viable source of nutrients, reducing the costs generated by the substrates acquisition or raw materials for their formulation, in addition to minimizing the environmental impact generated by inadequate residues disposal (Meneghelli et al., 2017).

Good management of composting combined with the use of suitable waste can produce organic composts with appropriate properties as a renewable substrate, promoting the best development of seedlings. Considering that the main commercial objective of horticultural activity is the production of standardized and healthy seedlings, the substrate must have physical, physical–chemical, and chemical properties with low variability (Pascual et al., 2018).

Regarding the improvement of nutrient cycling within rural properties, the millicomposting presents itself as a multifunctional alternative to family farming, conventional or organic, where agricultural residues can be transformed into quality organic composts to be used as substrate in seedling production (Antunes et al., 2020).

Unlike classic composting, which presents different temperature phases (mesophilic and thermophilic), millicomposting consist of a composting process without thermal variation, based on the diplopods' activity. Popularly known as millipedes, they act effectively in the process of decomposing plant residues into stable organic matter. This process is due to the type of oral apparatus of these arthropods, allowing them to crush the plant residues (litter) of carbon/nitrogen (C/N) ratios greater than 35 in smaller fragments (Aquino and Correia, 2005). Thus, there is the accelerating residues decomposition due to the increase of their contact surface, in addition to providing the association of other meso and microfauna organisms, which will contribute secondarily to the decomposition process.

The final product of millicomposting is millipede humus (millicompost), a stable organic compound with a C/N ratio $<$20, presenting quality to be used as a substrate. Although the knowledge regarding millicompost is scarce in the scientific and rural community, some results have shown that it provides excellent quality seedlings due to its physical and chemical characteristics suitable for plant development, such as vegetables (lettuce, arugula) and fruit tree (dragon fruit) (Antunes et al., 2019, 2018, 2021a,b; Bugni et al., 2021).

It is in family farming that most orchards are found, often located in small areas. This fact demands the need for research that can benefit and favor the small producer. Over the years, the passion fruit culture has shown itself as an alternative income for small and medium rural producers, due to the value of the commercialized fruits, harvested in a relatively short period when compared to other fruits requiring longer production (Santos et al., 2017).

In a previous study using the pure millicompost (without any other raw mixture) in fruit species, the pitaya (*Hylocereus undatus*), revealed that the millicompost physicochemical, chemical and physical properties were adequate for the development of pitaya cuttings, providing superior quality and size seedlings in relation to seedlings from other two substrates tested (sand plus manure and commercial organic substrate) (Antunes et al., 2021b).

Due to the excellent results with pitaya, and knowing the demand by seedling producers for quality substrates, we applied millicompost in the production of passion fruit seedlings, since researches reporting its use and benefits are scarce. In this context, the present study aimed (1) to formulate different organic substrates based on millicompost combined with different proportions of locally available organic residues, such as: coconut fiber (*Cocos nucifera*) powder, branches and leaves of gliricidia (*Gliricidia sepium*) and the elephant grass (*Pennisetum purpureum*) shavings, in order to seek the maximization of the use of millicompost in rural properties; (2) to characterize the physical, physical–chemical and chemical properties and (3) to evaluate the efficiency of these formulated substrates in the production of yellow passion fruit seedlings, in order to have new options for sustainable substrates for producers.

Table 1
PH values, electrical conductivity (EC), C/N ratio and levels of macronutrients present in the residues used in the formulation of different substrates with the millicompost.

Organic residue	pH	EC (dS m^{-1})	C/N ratio	N	P	K	Ca	Mg
				(g kg^{-1})				
Elephant grass	11.15	1.56	36.57	9.92	4.12	32.17	3.95	1.66
Coconut fiber	5.96	0.90	63.40	6.46	4.63	12.33	12.06	2.96
Gliricidia	9.20	1.04	30.15	13.44	1.88	18.65	12.53	2.88

Values obtained according to the methodology described by Teixeira et al. (2017).

2. Material and methods

2.1. Millicompost production

The millicompost was produced in field conditions close to a small secondary forest (Fig. S1), in Seropédica, Rio de Janeiro, Brazil. Plant residues from gardening, such as tree pruning used in urban landscaping (*Ficus sp*., *Khaya ivorensis* (A. Chev.), *Syzygium cumini* (L.)) and grass clippings that constituted the millicomposting process were accumulated in a pile approximately 1.50 m high. The millipedes (diplopods) that acted in the decomposing process (Fig. S1) belonged to the two species: *Trigoniulus corallinus* (Gervais, 1847) - originally from Southeast Asia, but it is currently found in North and South America and African continent. The individuals from this species are easily found in anthropized areas rich in organic materials and humid; *Rhinocricus padbergi* (Verhoeff, 1938) - native to the Atlantic Forest, known as the giant millipede, easily found in rural and urban environments related to the Atlantic Forest fragments. The millipedes were naturally introduced into the waste pile, all from the secondary forest, which is next to the millicomposting area, so there was no need to collect them. If there is a need to collect millipedes, the use of protective gloves was always recommended, since during collection the arthropods can be stressed and release a liquid with a strong and pigmented odor, which can color the hands for several days, giving an appearance of necrotic skin although, it does not offer health risk.

The millicompost was obtained 120 days after the beginning of the millicomposting, being removed and sieved in a 2 mm mesh (Fig. S1) to make it homogeneous for the use as a substrate.

2.2. Experimental conditions

The experiment with passion fruit seedlings was carried out in a greenhouse on the premises of Embrapa Agrobiologia, located in Seropédica-RJ, from May 27 to July 6, 2019. The altitude of the place is 33 m and the climate is classified as humid tropical (Aw), with an average annual precipitation of 1213 mm concentrated from November to March and an average annual temperature of 24.5 °C (Oliveira Júnior et al., 2014).

The substrates were formulated from renewable organic sources: coconut fiber (*Cocos nucifera*) in commercially acquired powder; leaves and thin branches of gliricidia (*Gliricidia sepium*) and elephant grass shavings (*Pennisetum purpureum*). These last two residues were processed in a mincer to obtain particles of $\pm$ 2 cm in diameter and then were spread on a canvas, forming a layer of 10 cm, where they remained in the shade for 30 days, being turned over every 10 days to speed up the drying process. The pH values, electrical conductivity (EC), C/N ratio and nutrient content of the residues are contained in Table 1.

The treatments consisted of six organic substrates: S1- millicompost; S2- commercial substrate (based on composted pine bark); S3- 25% millicompost + 25% gliricidia + 25% elephant grass + 25% coconut fiber; S4- 50% millicompost + 50% elephant grass; S5- 50% millicompost + 50% gliricidia; S6- 50% millicompost + 50% coconut fiber (Fig. S2). The formulations were prepared (v: v), quantified and added in a concrete mixer for better particles homogenization and later use as a substrate.

2.3. Analysis of physical, physical–chemical and chemical properties of substrates

The physical characteristics evaluated in the organic substrates were the following: macroporosity, microporosity, total porosity, water retention capacity and volumetric density (Teixeira et al., 2017).

To characterize the substrates in terms of their physical–chemical characteristics, pH analysis was performed in a distilled water solution (5: 1 v/v) and the electrical conductivity was determined in the same aqueous extract obtained from pH measurement, according to with the method described by Brasil (2008). As for the chemical properties, samples of each organic substrate were sent to the Agricultural Chemistry Laboratory of Embrapa Agrobiologia to determine the total and available levels of N, P, K, Ca and Mg.

The total levels of Nitrogen (N), Calcium (Ca), Magnesium (Mg), Potassium (K) and Phosphorus (P) were evaluated by means of sample digestion, according to the method described by Teixeira et al. (2017). The available levels of N, Ca, Mg, K and P were evaluated by means of extraction. For Ca and Mg, 1.0 M KCl extracting solution was used and for K and P, Mehlich 1 extracting solution was used, as described by Teixeira et al. (2017). The extraction of *N* was performed using a 1.0 M KCl solution and digestion was performed with the addition of Devarda's League, according to the methodology described by Liao (1981).

2.4. Morphological evaluations of passion fruit seedlings

The substrates, in 500 mL, were placed in black polyethylene bags measuring 10 × 20 cm, where the yellow passion fruit (*Passiflora edulis* Sims f. *Flavicarpa* Deg.) was sown using two seeds in each container.

After germination, thinning was done, leaving only one more vigorous seedling per container and at 56 days of sowing, the following morphological parameters were evaluated: shoot dry mass; dry root mass; plant height (cm), which was measured with a ruler from the lap to the apex of the plant (terminal bud); the number of leaves; stalk diameter (mm), measured at 1 cm from the substrate, with the aid of a digital caliper with a precision of 0.01 mm; root volume, obtained by displacing the water column contained in a 50 mL measuring cylinder; leaf area in cm^2, obtained through the LICOR leaf area meter (LI-3000); seedling vigor and clod stability. For the determination of dry masses, the shoot and roots of the plants were packed separately in paper bags and kept in a forced air circulation oven at 65 °C for 72 h and their respective masses were measured on a precision scale (0,01 g) later.

The contents of total macronutrients (N, P, K Ca and Mg) contained in the shoot of the plants collected were determined from the grinding of the shoot dry mass of the plants in Willey knife mill (Teixeira et al., 2017). To establish the quantities of each nutrient accumulated per plant, the value of the contents of each nutrient (g kg^{-1}) was multiplied by the dry mass of the aerial part of the plant, obtaining the data of macronutrient accumulation in mg $plant^{-1}$.

Seedling vigor is a methodology adapted from Antunes et al. (2018), classifying as: Note 1: excellent vigor, number of leaves ≥7, a height greater than 25 cm, may show photosynthetically active cotyledon leaves and visual absence of nutritional deficiency; Note 2: good vigor, number of leaves ≥6, a height between 20 and 25 cm and initial yellowing and/or absence of cotyledon leaves and/or yellowing of two true leaves; Note 3: regular vigor, number of leaves ≤5, a height between 15 to 20 cm; pronounced yellowing of two or more true leaves; Note 4: poor vigor, well-defined nutritional deficiency, expressed by problems with seedling height (<15 cm) and leaves expansion.

Clod stability is a methodology adapted from Antunes et al. (2018), classifying as: Note 1: excellent stability, the clod is removed from the plastic bag with 90% cohesion and the maximum losses of substrates are less than 10%, preserving the integrity of the root system; Note 2: good stability, the clod shows cohesion when removed from the bag between 70 and 90% and the losses of substrate are between 10 and 30%, leaving the root system partially exposed; Note 3: regular stability, the clod removed from the plastic bag shows cohesion between 50 and 70% and there are losses of substrate between 30 and 50%, leaving the root system clearly exposed; Note 4: low stability, the clod is removed from the bag showing less than 50% cohesion and the substrate losses are greater than 50%, leaving the root system exposed in proportion to the losses, which will cause damages in the establishment of the seedling in the field.

2.5. Statistical analysis

The experimental design adopted was randomized blocks with six treatments (substrates) and four replications, where each block consisted of eight seedlings per substrate, totaling 192 experimental units (seedlings). For the statistical analysis of the data generated, the homogeneity of the error variances was verified by the Bartlett test and normality by the Shapiro–Wilk test. Subsequently, the data were subjected to analysis of variance with the application of the Scott–Knott test ($p \leq 0.05$), using the statistical program Rbio (Bhering, 2017). The principal component analysis method (PCA) was employed by the PAST statistical program (Hammer et al., 2020) to assess the influence of different substrate formulations on the morphological parameters of passion fruit seedlings.

3. Results and discussion

3.1. Physical properties of organic substrates

The percentages of macropores differed between the substrates, the S1- millicompost showed higher macroporosity, followed by the commercial substrate. The other substrates registered percentages varying from 8 to 18% (Table 2). For microporosity, the millicompost had the lowest percentage, followed by the commercial substrate and S6. For the other substrates (Table 2), the microporosity range varied from 69 to 80%. Total porosity, on the other hand, showed percentages ranging from 71 to 90%. The water retention capacity (WRC) was higher for three of the four formulated substrates (S3, S4 and S5) and S6 registered the lowest WRC (Table 2). The volumetric density (VD) was different between the substrates, and the millicompost (S1) presented the highest VD. The other substrates showed VD ranging from 0.18 to 0.27 kg m^{-3} (Table 2).

Gonçalves and Poggiani (1996) consider the 35%–45% range to be adequate for macroporosity. However, in the present study, only the millicompost met this parameter, as the commercial substrate presented macroporosity considered average (20%–40%) by the authors and all formulated substrates presented macroporosity below the proposed range, varying from 8 to 18%. For microporosity, the aforementioned authors consider the range between 45%–55% to be adequate, however, none of the six substrates evaluated here reached this range, the millicompost presented the range considered average (25%–50%) and the other substrates remained within the range considered high (>55%). Microporosity is responsible for retaining water in the substrate (Maggioni et al., 2014) and according to Sá et al. (2020), substrates produced from organic residues have a predominance of micropores, corroborating the results obtained for substrates S3, S4, S5 and S6 (Table 2).

Table 2
Average values of macroporosity (MAC), microporosity (MIC), total porosity (TPO), water retention capacity at 10 cm of water column (WRC_{10}) and volumetric density (VD) from different organic substrates formulated based on millicompost to produce yellow passion fruit seedlings.

Substrates	MAC	MIC	TPO	WRC_{10} (mL 50 cm^3)	VD (g cm^{-3})
		(%)			
S1	43.45	39.64	83.09	37.14	0.40
S2	27.48	58.33	85.81	35.55	0.21
S3	8.84	80.75	89.59	41.18	0.19
S4	15.36	75.56	90.92	42.67	0.27
S5	18.07	69.48	87.55	39.41	0.25
S6	10.11	61.16	71.27	30.84	0.18

S1- millicompost; S2- commercial substrate; S3- 25% millicompost + 25% gliricidia + 25% elephant grass + 25% coconut fiber; S4- 50% millicompost + 50% elephant grass; S5- 50% millicompost + 50% gliricidia; S6- 50% millicompost + 50% coconut fiber.

Table 3
PH values and electrical conductivity (EC) of the organic substrates formulated based on millicompost to produce yellow passion fruit seedlings.

Substrates	pH	EC (dS m^{-1})
S1	5.87	0.94
S2	4.64	0.39
S3	8.19	0.81
S4	8.78	0.99
S5	9.06	1.06
S6	6.50	0.66

S1- millicompost; S2- commercial substrate; S3- 25% millicompost + 25% gliricidia + 25% elephant grass + 25% coconut fiber; S4- 50% millicompost + 50% elephant grass; S5- 50% millicompost + 50% gliricidia; S6- 50% millicompost + 50% coconut fiber.

Total porosity is extremely important for plant growth (Kämpf, 2005), since the high concentration of roots formed in the containers requires a high oxygen supply and rapid removal of the formed carbon dioxide, whose recommended percentages vary from 50 to 80% (Pascual et al., 2018), with only the substrate S6 met this recommendation (Table 2).

Gonçalves and Poggiani (1996) consider values between 20–30 mL 50 cm^{-3} to be adequate levels of water retention capacity (WRC). In this way, only the substrate S6 is within the appropriate range. All other substrates showed high WRC (>30 mL 50 cm^{-3}), as established by these authors. Such results are due to the high microporosity observed in the substrates S2 to S6 (Table 2). The higher WRC in organic compounds becomes an advantage in relation to the use of soil, as it reduces the frequency of irrigation shifts (Sá et al., 2020).

The volumetric density of organic composts intended for use as an organic substrate must be in the range of 0.30 to 0.75 g cm^{-3} (Pascual et al., 2018). Fermino (2014), with greater specificity, established as a reference for substrates used in trays, volumetric density values between 0.10 and 0.30 g cm^{-3}. In this way, all substrates can be used in trays, except for S1- millicompost, which was 33% above the reference according to the last author mentioned above. The fact that the millicompost was produced in the opened and the aboveground soil, at the time of its removal, the addition of soil may have occurred, which certainly caused it to become denser in relation to previous results, whose density was 0.29 g cm^{-3} in protected production (Antunes et al., 2020).

Volumetric density is extremely important to interpret other substrate properties, such as porosity, aeration space and water availability (Fermino, 2014). In addition, knowledge of its value has several applications to cultivation in containers, serving as a parameter for the management of irrigation and in the analysis of nutrients (regarding sample mass), which is indispensable for interpretation reports and practical recommendations (Fermino and Kämpf, 2012).

3.2. Physico-chemical properties of organic substrates

The pH values of organic substrates ranged from 4.64 (S2) to 9.06 (S5) (Table 3). According to Ludwig et al. (2014), pH value is more important than the substrate's own nutrient content, since it mainly affects the absorption of macro and micronutrients. pH values below 5.8 can lead the plant to toxicity, as it increases the availability of iron and manganese, in addition to reducing the availability of nitrogen, potassium, calcium, magnesium and boron (Stöcker et al., 2016). While pH values above 6.5, deficiencies of phosphorus, iron, manganese, zinc and copper can be expected (Kratz et al., 2014). Thus, only the millicompost (S1) and the substrate S6 remained in the range proposed by the aforementioned authors.

The raw material that makes up the substrate has a direct influence on pH values. The pine bark is naturally acidic and the pH value of the substrate S2 corroborates with the results obtained by Ludwig et al. (2020), whose values varied between 4.5 and 4.6 in substrate based on pine bark with different particle sizes. For the substrates S3, S4, and S5, the predominance of elephant grass and gliricidia caused an increase in pH values, making these substrates more alkaline. Alkalization is due to the chemical constitution of these raw materials, whose main form of nitrogen (N) is in the protein form *N* (Silva et al., 2013). Probably because they were only air-dried and not composted, the acidification induced by

Table 4
Total contents, available and proportion of available contents of macronutrients in organic substrates formulated based on millicompost to produce yellow sour passion fruit seedlings.

Substrates	N	P	K	Ca	Mg
	Total contents (g kg^{-1})				
S1	16.56	1.57	3.17	20.15	3.23
S2	10.41	2.15	2.46	5.23	1.24
S3	13.91	2.45	14.24	17.65	2.67
S4	14.87	2.43	10.75	22.81	3.52
S5	17.32	2.22	7.93	26.31	4.75
S6	13.15	1.71	7.09	19.42	2.92
Substrates	N	P	K	Ca	Mg
	Available contents (g kg^{-1})				
S1	2.25	0.77	3.17	12.12	2.21
S2	3.88	1.57	2.02	4.78	1.06
S3	2.35	1.76	12.88	9.88	2.04
S4	2.37	2.06	10.75	10.39	1.92
S5	2.72	1.50	7.93	11.42	2.32
S6	2.55	1.37	7.09	11.81	2.20
Substrates	N	P	K	Ca	Mg
	Proportion of available contents (%)				
S1	14	49	100	60	68
S2	37	73	82	91	85
S3	17	72	90	56	76
S4	16	85	100	46	55
S5	16	68	100	43	49
S6	19	80	100	61	75

S1- millicompost; S2- commercial substrate; S3- 25% millicompost + 25% gliricidia + 25% elephant grass + 25% coconut fiber; S4- 50% millicompost + 50% elephant grass; S5- 50% millicompost + 50% gliricidia; S6- 50% millicompost + 50% coconut fiber.

the transformation of N-ammonium (NH_4^+) into N-nitrate (NO_3^-) did not occur (Leal et al., 2013). Higher values of pH, especially in the substrates S4 and S5, whose proportions of elephant grass and gliricidia were 50%, favored the ammonium production (NH_4^+) through the mineralization of *N* present in the organic form. Likely, the nitrification process was not so expressive, therefore the reduction in pH was not observed in these substrates. Another factor that contributed to the higher pH values is the pH values of elephant grass (11.15) and gliricidia (9.20) (Table 1), corroborating with the results observed by Silva et al. (2013) when evaluating the quality of organic compounds produced with different proportions of crushed branches of gliricidia and elephant grass.

The electrical conductivity (EC) varied from the organic substrates between 0.39 dS m^{-1} (S2) and 1.06 dS m^{-1} (S5) (Table 3). The EC indicates the concentration of salts present in the solution (distilled water + substrate) and provides an estimation of salinity present in the substrates. Moreover, it infers, in a practical way, that the substrates S5, S4, and S1 presented higher nutrient inputs. According to Minami and Salvador (2010), EC values above 3.4 dS m^{-1} are considered too high for substrates, values from 2.25 to 3.39 dS m^{-1} are high, values from 1.8 to 2,24 dS m^{-1} are slightly high, values from 0.5 to 1.79 dS m^{-1} are moderate, values between 0.15 and 0.49 dS m^{-1} are low and values below 0.14 dS m^{-1} are considered very low. In this way, all substrates showed moderate EC, except S2, which exhibited an EC considered low.

3.3. Chemical properties of organic substrates

The levels of total and available nutrients, and the available proportions of N, P, K, Ca and Mg contained in the evaluated substrates are shown in Table 4. Concerning the N levels, which is generally the most demanded nutrient for the seedlings, the contents in the substrates ranged from 10.40 g kg^{-1} (S2) to 17.32 g kg^{-1} (S8). For the levels of available nutrients, the substrate S7 showed highest contents for phosphorus (P) and potassium (K), the millicompost showed more calcium (Ca), and S8 was the substrate with more content of magnesium (Mg). In relation to the level of available N, the commercial (S2) substrate showed the higher content. For the substrates formulated based on millicompost, S5 and S6 showed the highest availability of N, Ca and Mg (Table 4).

According to Pereira et al. (2020), there is no current information on the adequate levels of nutrients contained in substrates for plants. However, Gonçalves and Poggiani (1996) established value scales for the interpretation of the substrates chemical characteristics, such as the adequate levels of macronutrients: phosphorus (P), potassium (K), calcium (Ca), and magnesium (Mg).

The substrate is essential in the production chain of vegetables that are grown through seedlings and for quality seedlings to be produced, it must contain chemical properties suitable for plant development. Nitrogen is the nutrient that influences most of the physiological processes that occur in plants, such as protein synthesis and photosynthesis,

Table 5
Organic carbon content and carbon/nitrogen ratio (C/N) of the organic substrates formulated based on millicompost to produce yellow passion fruit seedlings.

Substrates	Organic carbon (%)	C/N ratio
S1	33	19.92
S2	38	36.86
S3	34	24.17
S4	28	18.74
S5	30	17.07
S6	31	23.78

S1- millicompost; S2- commercial substrate; S3- 25% millicompost + 25% gliricidia + 25% elephant grass + 25% coconut fiber; S4- 50% millicompost + 50% elephant grass; S5- 50% millicompost + 50% gliricidia; S6- 50% millicompost + 50% coconut fiber.

being the most limiting nutrient in biomass production (Yong et al., 2010), essential in the production phase of seedlings. The proportion of available N was higher in the commercial substrate (S2) and in the other substrates the percentages varied from 14 to 19% (Table 4).

Phosphorus has important structural functions for plant development, participating in photosynthesis, respiration, division, and cell growth, and especially in energy supply (ATP), providing greater growth and initial development of plants, especially the root system (Berti et al., 2017). The levels of phosphorus considered adequate vary in the range of 0.4 to 0.8 g kg^{-1} (Gonçalves and Poggiani, 1996), although here, the available levels have been well above from this range for all substrates (Table 4).

According to Gonçalves and Poggiani (1996), it is considered adequate for potassium contents levels between 1.17 to 3.91 g kg^{-1}. In this case, only the commercial substrate (S2) matched the range considered adequate and the other substrates had available contents higher than those proposed by the authors, mainly for S3 and S4 (Table 4), due to the types of raw materials used (Table 1), rich in this nutrient. Unlike nitrogen and phosphorus, potassium does not have a structural function, however, it is associated with greater resistance of plants when subjected to adverse conditions, such as low water availability and extreme temperatures, due to its function in opening and closing stomata (Berti et al., 2017). However, none of these adverse conditions were recorded during the development of passion fruit seedlings.

The levels of calcium considered adequate vary from 2 to 4 g kg^{-1} (Gonçalves and Poggiani, 1996). In the present study, all the substrates had levels of available Ca above the adequate levels, ranging from 4.78 to 11.81 kg^{-1} among the substrates S2 to S6. Nevertheless, the millicompost (S1) exhibited calcium content 2.5 times greater than substrate S2. Calcium is a fundamental element in membrane permeability and maintenance of cell integrity, being required for the division and expansion of cells. It is a component of the cell wall and middle lamella, and also serves as an activator for some enzymes involved in carbohydrate metabolism, such as alpha-amylase (Garrone et al., 2016).

Regarding the levels of available magnesium, all other substrates exhibited Mg contents below the range considered adequate, which according to Gonçalves and Poggiani (1996) varies from 6 to 12 g kg^{-1}. Among the functions of magnesium, its role in the composition of the chlorophyll molecule stands out, participating in several processes, such as photosynthesis, respiration, synthesis of carbohydrates and proteins (Silva et al., 2017).

Dias et al. (2010) claim that the longer composting time promotes a decrease in the organic matter (OM) content of the compost, and the organic carbon content (C) is a great parameter to assess the degree of humification inorganic composts. Schmitz et al. (2002) reported that 50 to 60% of the organic matter consists of carbon and the ideal levels of organic carbon for substrates used in containers should be above 25%, thus, all the substrates used in this study are appropriate, presenting content organic carbon ranging from 27 to 38% (Table 5).

The carbon/nitrogen ratio (C/N) is essential for the substrates characterization, signaling how the organic composts will be found at the end of the composting process (Da Ros et al., 2015), besides, it is indispensable when there are no others robust types of analysis to ascertain the stability of the organic compost. The Normative Instruction Nº 61 from Ministry of Agriculture, Livestock and Food Supply (Brasil, 2020) highlights that the C/N ratio cannot be higher than 20 and the total nitrogen content must be at least 5.0 g kg^{-1} for organic composts. Therefore, only the substrates S2, S3 and S6 exhibited C/N ratios above 20 (Table 5), a result of their formulations, since the substrate S2 was composed of pine bark, corroborating with the results found by Antunes et al. (2019), who also observed a high C/N ratio for this type of substrate. For the other substrates, formulated with a mixture of elephant grass, gliricidia, coconut fiber, and millicompost (S3) and millicompost combined with coconut fiber (S6), it remained outside the recommended range due to the C/N ratio of the coconut fiber (63), while the C/N ratios of elephant grass and gliricidia are half their value (Table 1). Regarding the nitrogen content, all the evaluated substrates showed levels above the minimum recommended by the normative instruction.

3.4. Morphological parameters of passion fruit seedlings

The organic substrates formulated based on millicompost provided yellow passion fruit seedlings with significantly different morphological characteristics ($p < 0.05$) for all the phytotechnical parameters evaluated (Fig. 1).

The dry mass of shoots ranged from 0.51 g to 1.65 g (Fig. 1a), which was significantly higher in the substrates S1 (millicompost), S6 (50% millicompost + 50% coconut fiber), and S5 (50% millicompost + 50% gliricidia). These substrates

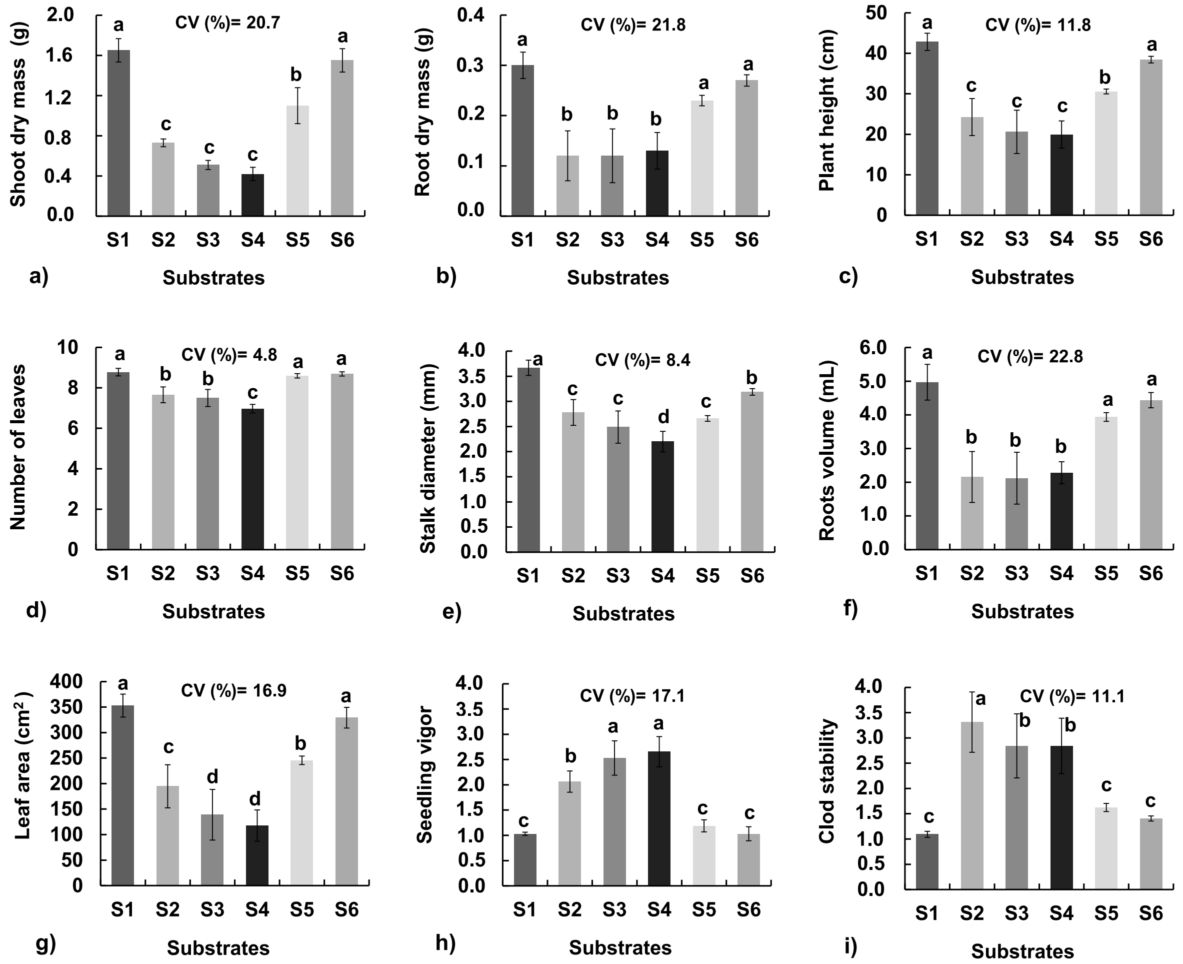

Fig. 1. Values of shoot dry mass, root dry mass, plant height, number of leaves, stalk diameter, volume of roots, leaf area, seedling vigor and clod stability of yellow sour passion fruit seedlings produced in different organic substrates formulated based on millicompost. Means followed by the same letter in the bars do not differ from each other by the Scott–Knott test ($p < 0.05$). S1- millicompost; S2- commercial substrate; S3- 25% millicompost + 25% gliricidia + 25% elephant grass + 25% coconut fiber; S4- 50% millicompost + 50% elephant grass; S5- 50% millicompost + 50% gliricidia; S6- 50% millicompost + 50% coconut fiber.

also provided the greatest gains in root dry matter, being statistically superior to the other substrates (Fig. 1b). The dry mass values found in the aforementioned substrates were similar to those found by Dantas et al. (2015), however, the authors used soil enriched with bovine manure at a concentration of 50%. The production of dry matter is associated with the accumulation of nutrients extracted by the plant during its development, so it is possible to know which substrate provided greater quantities of nutrients to the passion fruit seedlings. Moreover, the shoot dry mass is an important growth parameter, directly associated with field crop productivity (Azevedo et al., 2020; Costa et al., 2013).

The height of the plants ranged from 19.95 to 42.84 cm (Fig. 1c), being significantly higher in seedlings grown on the substrates S1 (millicompost), S6 (50% millicompost + 50% coconut fiber), and S5 (50% millicompost + 50% gliricidia). Although the formulated substrates S3 (25% millicompost + 25% gliricidia + 25% elephant grass + 25% coconut fiber) and S4 (50% millicompost + 50% elephant grass) have provided seedlings with heights lower than the others, they match the results obtained by Cordeiro et al. (2019) for passion fruit seedlings produced in commercial substrate evaluated at 60 days after sowing. According to Bertani et al. (2019), the seedlings from the conventional system are developed in tubes and, in this condition, they are taken to the field approximately 30 days after sowing, being less vigorous and with an average height of 15 to 30 cm.

The largest number of leaves (eight units) was observed in the seedlings developed on the substrates S1 (millicompost), S5 (50% millicompost + 50% gliricidia), and S6 (50% millicompost + 50% coconut fiber), which were significantly larger in relation to the seedlings of the other substrates (Fig. 1d). Our results corroborate those found by Dantas et al. (2015), which used soil enriched with bovine manure at a concentration of 50%. The number of leaves is a good indicator of

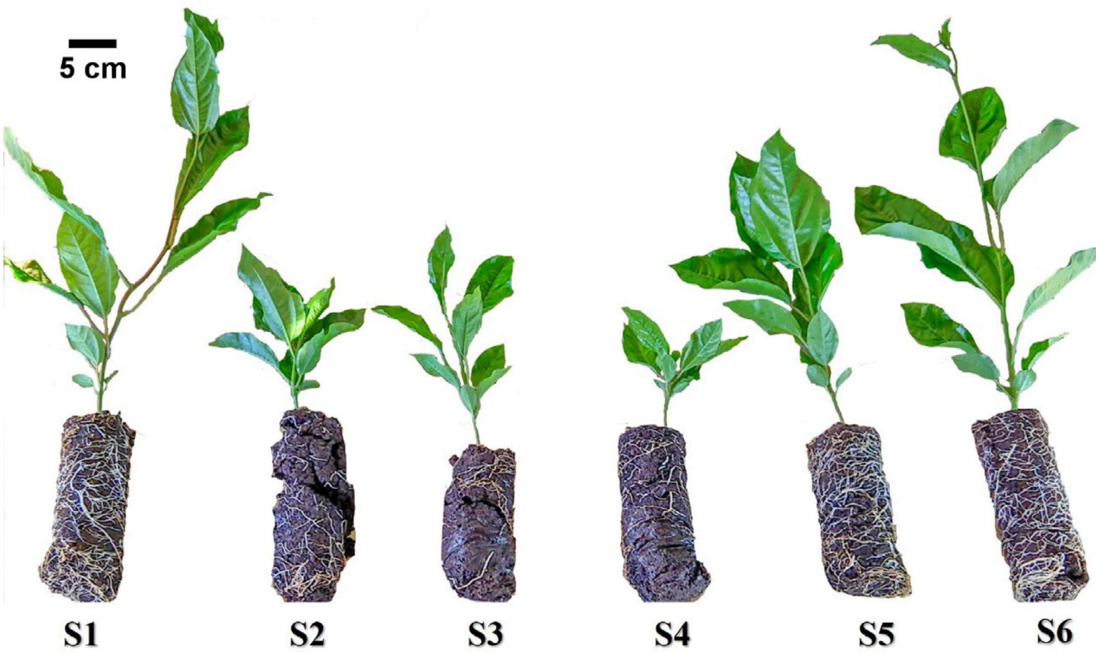

Fig. 2. Clear differences between the yellow passion fruit seedlings at 56 days after sowing, which were developed in different organic substrates: S1- millicompost; S2- commercial substrate; S3- 25% millicompost + 25% gliricidia + 25% elephant grass + 25% coconut fiber; S4- 50% millicompost + 50% elephant grass; S5- 50% millicompost + 50% gliricidia; S6- 50% millicompost + 50% coconut fiber.

seedlings vigor, reflecting on the its performance under natural conditions, since more vigorous seedlings have lower mortality rates and its establishment is faster (Miyake et al., 2017).

The largest stalk diameters (>3 mm) were observed in the passion fruit seedlings from the substrates S1 (millicompost) and S6 (50% millicompost + 50% coconut fiber) (Fig. 1e). These results are similar to those found by Cordeiro et al. (2019) when producing *Passiflora edulis* seedlings in substrate formulated with 80% decomposed babassu stalk and 20% soil plus sand (1: 1). The substrate that provides seedlings with a balance between the growth of stalk diameter and height also provides greater strength and greater resistance to adverse conditions found in the field, such as the action of strong winds. In this way, survival rates are raised and production costs are reduced (Azevedo et al., 2020; Smiderle et al., 2020).

The root volume (Fig. 1f) varied between the substrates and was significantly higher in the seedlings from the substrates S1 (millicompost), S6 (50% millicompost + 50% coconut fiber), and S5 (50% millicompost + 50% of gliricidia). Cordeiro et al. (2019), when producing *Passiflora edulis* seedlings in substrate formulated with 80% decomposed babassu stalk and 20% soil plus sand (1: 1), obtained root volume similar to substrate S6. The results of root volume are relevant, considering that the greater volume of roots provides a greater amount of nutrients that will be absorbed between transplantation and the formation of new roots, directly influencing the final performance of plants (Azevedo et al., 2020).

The leaf area of the seedlings grown on the substrates S1 (millicompost), S6 (50% millicompost + 50% coconut fiber), and S5 (50% millicompost + 50% gliricidia) were significantly larger in relation to the other substrates (Fig. 1g). The reduced leaf areas observed in substrates S2 (commercial substrate); S3 (25% millicompost + 25% gliricidia + 25% elephant grass + 25% coconut fiber) and S4 (50% millicompost + 50% elephant grass) are related to a lower number of leaves and lower leaf blade, which can also be explained by the unfavourability of these substrates, mainly regarding pH values, discussed previously in item 3.2 (Table 3). Souza et al. (2014) emphasize the importance of leaves in the vegetal production, considering the leaf area as an index of productivity, influencing the photosynthetic capacity, especially in the development of the plant's vegetative compartment (Miyake et al., 2017).

The quality of the seedlings that will be transferred to the production fields can be expressed by the seedling vigor and stability of the root ball. Although many studies use the Dickson quality index (DQI), we consider that visual evaluation according to predetermined morphological parameters is practical, innovative, and an easy methodological transfer to the producers. The better notes of seedling vigor and clod stability were provided by the substrates S1 (millicompost), S6 (50% millicompost + 50% coconut fiber), and S5 (50% millicompost + 50% gliricidia), where the seedlings received note1, showing the efficiency of these substrates in producing quality seedlings (Figs. 1h, i and 2). The other substrates provided seedlings with lower size, morphology, and clod stability (Fig. 2).

According to Menegaes et al. (2017), the substrate used for the production of seedlings has a direct relationship with the formation and clod stability, providing ideal conditions for root development. Besides, its integrity is essential for the

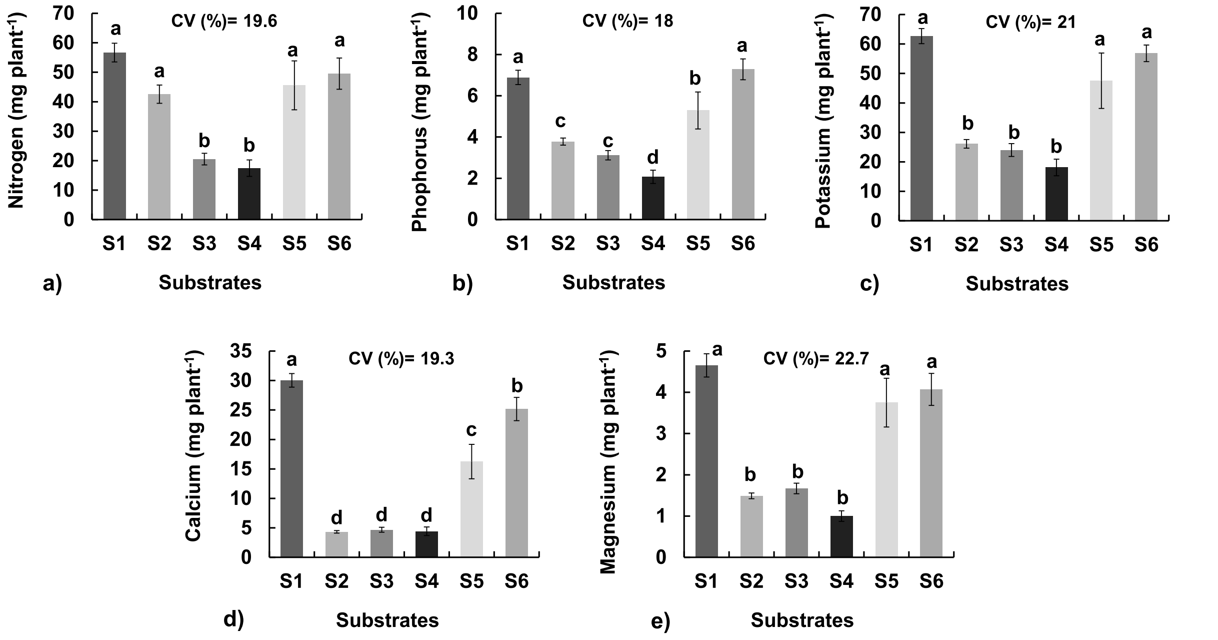

Fig. 3. Total nutrient contents accumulated in the shoot dry mass of the yellow sour passion fruit seedlings produced in the different organic substrates formulated based on millicompost. Means followed by the same letter in the bars do not differ from each other by the Scott–Knott test ($p < 0.05$). S1- millicompost; S2- commercial substrate; S3- 25% millicompost + 25% gliricidia + 25% elephant grass + 25% coconut fiber; S4- 50% millicompost + 50% elephant grass; S5- 50% millicompost + 50% gliricidia; S6- 50% millicompost + 50% coconut fiber.

successful establishment of seedlings in the field, as an unstable clod promotes disruption and exposure of the root system, leaving the plant more vulnerable to desiccation and death, requiring replanting actions or leading to late production cycle.

The nutrient contents accumulated in the aerial part of the passion fruit seedlings were significantly influenced by ($p < 0.05$) the different substrates (Fig. 3). The substrates S1 (millicompost), S6 (50% millicompost + 50% coconut fiber) and S5 (50% millicompost + 50% gliricidia) promoted the largest nutrients accumulation of, following the order K > N > Ca > P > Mg. Cordeiro et al. (2019), when evaluating different proportions of decomposed babassu stem combined with soil and sand (1:1), registered similar results only for nitrogen accumulation (50 mg plant^{-1}) in substrates with 100% decomposed babassu stem and on the commercial substrate.

The relationship between the variables of seedlings growth and quality in different organic substrates can be visualized throughout the principal component analysis (PCA). The data variability was explained by 93.69% on axis 1 and 3.83% on axis 2, totaling 97.52% of all data variability (Fig. 4). These results are in agreement with Rencher and Christensen (2012), who suggest that the number of main components used in the interpretation must have an explanation level of at least 70% of the total variance of the original data.

It is possible to observe that the variables were separated into two groups of substrates. The first was comprised by substrates S1 (millicompost), S6 (50% millicompost + 50% coconut fiber) and S5 (50% millicompost + 50% gliricidia), corroborating with the results obtained through univariate statistics (Figs. 1 and 2), which positively influenced on morphological characteristics of SDM; RDM; PH; NL; SD; RV; LA; SV, and CS (Fig. 4). The second group included the substrates S2 (commercial), S3 (25% millicompost + 25% gliricidia + 25% grass + 25% coconut fiber) and S4 (50% millicompost + 50% elephant grass), which are correlated with the CS and SV variables (Fig. 4).

In general, these results confirm the importance of using a quality organic compost in the composition of formulated substrates for the production of *Passiflora edulis* seedlings. Organic matter improves the physical and chemical properties of substrates and increases the availability of macronutrients, micronutrients, and plant growth regulators; however, the compound represents by far the most thoroughly investigated constituent of the growing media (Pascual et al., 2018).

Previous research conducted in India by Ramanathan and Alagesan (2012) demonstrated that the millicompost generated at 60 days, from flower remnants, was the substrate that provided the highest plant height, number of leaves, leaf area, number and weight of fruits in peppers grown in pot, in relation to the plants developed in the vermicompost from the same residues of flowers. In Brazil, Antunes et al. (2016) found that the content of nutrients such as calcium, magnesium and phosphorus, as well as the physical–chemical and physical characteristics of the millicompost generated by millipedes of the species *Trigoniulus corallinus* were efficient in the production of lettuce seedlings.

Interestingly, the present study showed, for the first time, that passion fruit seedlings are produced in the millicompost. The results confirm its ability to promote excellent development of seedlings of the species *Passiflora edulis*. Even though

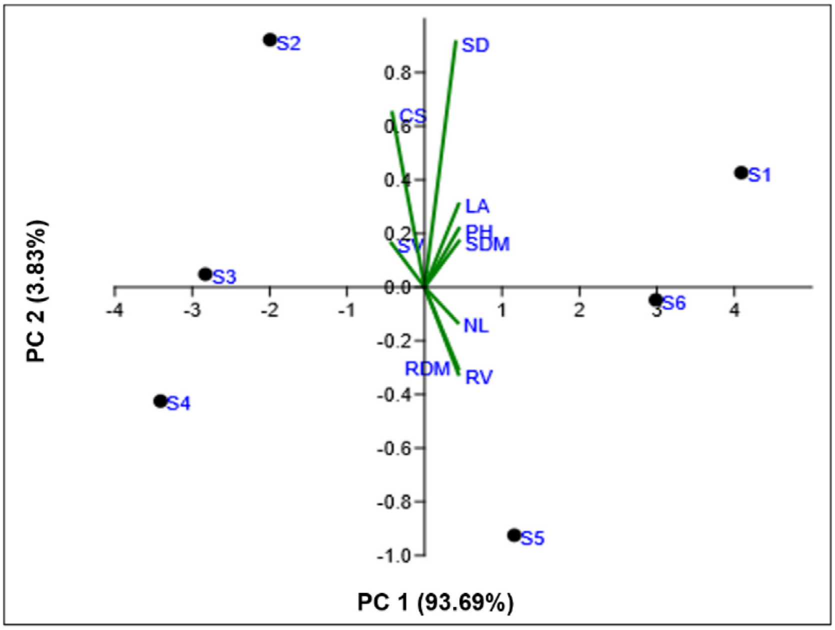

Fig. 4. Principal component analysis (PCA) related to the morphological parameters of yellow sour passion fruit seedlings produced in different organic substrates formulated based on millicompost. SDM = shoot dry mass; RDM = root dry mass; PH = plant height; NL = number of leaves; SD = stalk diameter; RV = root volume; LA = leaf area; SV = seedling vigor; CS = clod stability.

only 30% yield was acquired in the production of this compost, excellent results were achieved throughout combination with powdered coconut fiber and chopped and air-dried *Gliricidia sepium* for 30 days, thus providing two new substrate options to seedling producers, since the cultivation of passion fruit, for the most part, is carried out by family farmers, who seek alternative sources of cultivation and low cost (Santos et al., 2020).

Nevertheless, future research should investigate the influences of humid substances and the microbial community present in the millicompost, as well as in the formulated substrates, mainly the mixture of 50% millicompost and 50% powdered coconut fiber (S6), which presented smaller amounts of nutrients available in relation to the millicompost (S1), however, was efficient in producing seedlings similar in morphology and nutrient content in the shoot dry mass.

4. Conclusions

The millicompost was tested for the first time in the production of yellow passion fruit seedlings and demonstrated great efficiency, gathering desirable physical, physical–chemical, and chemical characteristics of a good substrate to obtain excellent quality seedlings.

The substrates formulated based on 50% millicompost with coconut fiber (S6), and 50% millicompost with 50% *Gliricidia sepium* (S5) are suitable to produce passion fruit seedlings and allow the maximization of the use of millicompost as a substrate.

Moreover, the millicompost used pure or combined with the aforementioned residues, represents a viable alternative for the production of sustainable organic substrates, mainly for the small producer, allowing the reuse of organic residues from the property and region. In addition, it generates more economy in acquisition of inputs, mainly when it refers of substrates that use anti-ecological components, such as peat, a non-renewable organic material and/or the use of soluble chemical fertilizers.

CRediT authorship contribution statement

Luiz Fernando de Sousa Antunes: Conceptualization, Methodology, Investigation, Formal analysis, data curation, Supervision, Writing – original draft, Writing – review & editing. **André Felipe de Sousa Vaz:** Investigation, Supervision, Writing – review & editing. **Luiz Aurélio Peres Martelleto:** Writing – review & editing. **Marco Antonio de Almeida Leal:** Writing – review & editing. **Renata dos Santos Alves:** Investigation, Writing – review & editing. **Talita dos Santos Ferreira:** Investigation, Writing – review & editing. **Norma Gouvêa Rumjanek:** Supervision, Writing – review & editing, Resources, Funding acquisition. **Maria Elizabeth Fernandes Correia:** Writing – review & editing, Resources, Funding acquisition. **Raul Castro Carriello Rosa:** Writing – review & editing, Resources, Funding acquisition. **José Guilherme Marinho Guerra:** Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary material related to this article can be found online at https://doi.org/10.1016/j.eti.2022.102612.

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